

What Is Claimed Is:

1. A modified mRNA molecule operable to crosslink to a tRNA molecule, wherein the modified mRNA molecule comprises a crosslinker located on or near a stop codon.
2. The modified mRNA molecule of Claim 1, wherein the crosslinker is an agent that can be activated to form one or more covalent bonds with the tRNA.
3. The modified mRNA molecule of Claim 1, wherein the crosslinker is an agent that is activated to form one or more covalent bonds with the tRNA using light.
4. The modified mRNA molecule of Claim 1, wherein the crosslinker is a modified base that is incorporated directly into the mRNA.
5. The modified mRNA molecule of Claim 1, wherein the crosslinker is selected from the group consisting of one or more of the following 2-thiocytosine, 2-thiouridine, 4-thiouridine, 5-iodocytosine, 5-iodouridine, 5-bromouridine and 2-chloroadenosine, aryl azides, and modifications or analogues thereof.
6. The modified mRNA molecule of Claim 1, wherein the crosslinker is psoralen.
7. A modified mRNA molecule operable to crosslink to a tRNA molecule, wherein the modified mRNA molecule comprises a crosslinker located on or near a pseudo stop codon.
8. The modified mRNA molecule of Claim 7, wherein the crosslinker is an agent that can be activated to form one or more covalent bonds with the tRNA.
9. The modified mRNA molecule of Claim 7, wherein the crosslinker is an agent that is activated to form one or more covalent bonds with the tRNA using light.
10. The modified mRNA molecule of Claim 7, wherein the crosslinker is a modified base that is incorporated directly into the mRNA.
11. The modified mRNA molecule of Claim 7, wherein the crosslinker is selected from the group consisting of one or more of the following 2-thiocytosine, 2-thiouridine, 4-thiouridine, 5-iodocytosine, 5-iodouridine, 5-bromouridine and 2-chloroadenosine, aryl azides, and modifications or analogues thereof.
12. The modified mRNA molecule of Claim 7, wherein the crosslinker is psoralen.

13. A kit to generate cognate pairs comprising at least one psoralen monoadduct attached to a nonadducted stable aminoacyl tRNA analog or at least one psoralen monoadduct attached to an oligonucleotide.

14. A method for evolving a desired protein sequence comprising:

- providing at least two candidate mRNA molecules, wherein at least one of said mRNA molecules contains at least one codon selected from the group consisting of a stop codon and a pseudo stop codon;
- translating at least two of said candidate mRNA molecules to generate at least one translated protein;
- linking at least one of said candidate mRNA molecules to its corresponding translated protein via a tRNA molecule to form at least one cognate pair, wherein at least one of said candidate mRNA molecules is connected to said tRNA molecule by a crosslinker;
- identifying one or more of said cognate pairs based upon the properties of said translated protein or said mRNA molecule;
- identifying a molecule selected from the group consisting of one or more of the following: an mRNA molecule of said selected cognate pair, a nucleic acid molecule complementary to said mRNA molecule and a nucleic acid molecule homologous to said mRNA molecule, thereby identifying said desired protein or said desired nucleic acid molecule;
- providing a plurality of cognate pairs,
- binding at least of said plurality of cognate pairs with one or more binding agents;
- selecting said desired or protein nucleic acid molecule based upon a reaction or lack of a reaction to said one or more binding agents, thereby selecting a first desired cognate pair;
- recovering said first desired cognate pair to generate a recovered cognate pair;
- amplifying a first nucleic acid component of said recovered cognate pair;

producing a second nucleic acid component, wherein said second nucleic acid component comprises said first nucleic acid component with one or more variations;

producing a second protein by translating said second nucleic acid component;

linking said second protein with said second nucleic acid component to generate a second desired cognate pair; and

obtaining the desired protein sequence by re-selecting said second desired cognate pair based upon at least one desired property.

15. The method of Claim 14, wherein said desired property is selected from the group consisting of one or more of the following: binding properties, enzymatic reactions and chemical modifications.

16. The method of Claim 14, wherein said desired property is an ability to resist binding, enzymatic reaction or chemical modification.

17. The method of Claim 14, wherein the step of selecting said first desired cognate pair comprises:

providing a first ligand with a desired binding characteristic;

contacting one or more of said first cognate pairs with said first ligand to generate unbound complexes and bound complexes;

recovering either the bound complexes or the unbound complexes;

amplifying at least one nucleic acid component of the recovered complexes;

introducing variation to a sequence of said nucleic acid component of said recovered complexes;

translating one or more second proteins from said nucleic acid components,

linking at least one of said second proteins with at least one of said second nucleic acid components to generate one or more second cognate pairs; and

obtaining the desired protein sequence by contacting said at least one of said second cognate pairs with at least one second ligand to select one or more

of said second cognate pairs, wherein said second ligand is the same or different than said first ligand.

18. A method of forming a psoralen monoadduct on a nucleic acid, comprising:

providing a first nucleic acid and a second nucleic acid,

wherein said first nucleic acid and said second nucleic acid are substantially complementary to each other,

wherein said first nucleic acid comprises one or more uridine monoadduct targets, and

wherein said second nucleic acid comprises at least one pseudouridine

hybridizing said first nucleic acid and said second nucleic acid in the presence of psoralen to form a hybrid;

irradiating said hybrid with ultraviolet light, thereby forming said psoralen monoadduct on said first nucleic acid.

19. The method of Claim 18, wherein said one or more uridine monoadduct targets comprises a uridine located adjacent to an adenosine.

20. The method of Claim 18, wherein said one or more uridine monoadduct targets comprises a uridine located adjacent to and 3' from an adenosine.

21. A method of producing a psoralen monoadduct or a crosslink, comprising:

providing a first nucleic acid and a second nucleic acid;

wherein said first nucleic acid and said second nucleic acid are substantially complementary to each other;

wherein said first nucleic acid comprises one or more uridine monoadduct targets or crosslink targets and one or more uridine monoadduct non-targets or crosslink non-targets;

wherein said uridine monoadduct non-targets or crosslink non-targets are operable to be replaced with one or more pseudouridines;

replacing one or more of said uridine monoadduct non-targets or crosslink non-targets with pseudouridine;

hybridizing said first nucleic acid and said second nucleic acid in the presence of psoralen to form a hybrid;

irradiating said hybrid, thereby forming said psoralen monoadduct or said crosslink on said first nucleic acid on said targets, while protecting said nontargets.

22. A method according to Claim 14, wherein the desired protein is one or more proteins of the SARS virus.

23. A method for selecting a desired protein or nucleic acid molecule comprising:

providing at least two candidate mRNA molecules, wherein at least one of said mRNA molecules contains at least one codon selected from the group consisting of a stop codon and a pseudo stop codon;

translating at least two of said candidate mRNA molecules to generate at least one translated protein;

linking at least one of said candidate mRNA molecules to its corresponding translated protein to form at least one cognate pair;

selecting one or more of said cognate pairs based upon the properties of said translated protein or said mRNA molecule; and

selecting said translated protein or said desired nucleic acid molecule comprising identifying a molecule selected from the group consisting of an mRNA molecule of said selected cognate pair, a nucleic acid molecule complementary to said mRNA molecule and a nucleic acid molecule homologous to said mRNA molecule.

24. A method for evolving a desired protein sequence comprising:

providing at least two candidate mRNA molecules, wherein at least one of said mRNA molecules contains at least one codon selected from the group consisting of a stop codon and a pseudo stop codon;

translating at least two of said candidate mRNA molecules to generate at least one translated first protein;

linking at least one of said candidate mRNA molecules to its corresponding translated first protein to form at least one first cognate pair;

selecting at least one of said first cognate pairs based upon at least one desired characteristic of said translated first protein or said mRNA;

recovering at least one of said first cognate pairs with said desired characteristic to generate at least one recovered cognate pair;

amplifying a first nucleic acid component of one or more of said recovered cognate pairs;

producing at least one second nucleic acid component comprising at least one of said first nucleic acid components with one or more variations;

producing at least one second protein by translating at least one of said second nucleic acid components;

linking at least one of said second proteins with at least one of said second nucleic acid components to generate one or more second cognate pairs; and

obtaining the desired protein sequence by re-selecting one or more of said second cognate pairs based upon at least one desired property, wherein said desired property is the same or different than said desired characteristic.

25. A method of forming a monoadduct comprising:

providing a target oligonucleotide comprising at least one uridine and at least one modified uridine,

contacting said target oligonucleotide with psoralen, and

coupling said psoralen to said target oligonucleotide to form a monoadduct.

26. A method for identifying and selecting a desired protein or nucleic acid molecule comprising:

providing at least two candidate mRNA molecules, wherein at least one of said mRNA molecules contains at least one codon selected from the group consisting of a stop codon and a pseudo stop codon;

translating at least two of said candidate mRNA molecules to generate at least one translated protein;

linking at least one of said candidate mRNA molecules to its corresponding translated protein via a tRNA molecule to form at least one

cognate pair, wherein at least one of said candidate mRNA molecules is connected to said tRNA molecule by a crosslinker;

identifying one or more of said cognate pairs based upon the properties of said translated protein or said mRNA molecule;

identifying a molecule selected from the group consisting of one or more of the following: an mRNA molecule of said selected cognate pair, a nucleic acid molecule complementary to said mRNA molecule and a nucleic acid molecule homologous to said mRNA molecule, thereby identifying said desired protein or said desired nucleic acid molecule;

providing a plurality of cognate pairs,

binding at said plurality of cognate pairs with one or more binding agents; and

selecting said desired protein or nucleic acid molecule based upon a reaction or lack of a reaction to said one or more binding agents.

27. The method of Claim 26, further comprising determining the DNA sequence of said translated protein, comprising:

providing an array of two or more DNA sequences, wherein said two or more DNA sequences are placed in a predetermined position;

exposing said array to said one or more cognate pairs, wherein said one or more cognate pairs comprises an mRNA portion and a protein portion;

hybridizing the mRNA portion of said one or more cognate pairs onto said array;

exposing the protein portion of said one or more cognate pairs to a binding agent, thereby producing a reaction or a non-reaction; and

selecting said desired protein based upon the reaction or non-reaction to said binding agent, thereby determining the DNA sequence of said translated protein.

28. A method for identifying a desired protein or nucleic acid molecule comprising:

providing at least two candidate mRNA molecules, wherein at least one of said mRNA molecules contains at least one codon selected from the group consisting of a stop codon and a pseudo stop codon;

translating at least two of said candidate mRNA molecules to generate at least one translated protein;

linking at least one of said candidate mRNA molecules to its corresponding translated protein via a tRNA molecule to form at least one cognate pair, wherein at least one of said candidate mRNA molecules is connected to said tRNA molecule by a crosslinker, wherein said tRNA molecule is a substantially unmodified native tRNA, and wherein said crosslinker is located only on at least one mRNA molecule;

identifying one or more of said cognate pairs based upon the properties of said translated protein or said mRNA molecule; and

identifying a molecule selected from the group consisting of one or more of the following: an mRNA molecule of said selected cognate pair, a nucleic acid molecule complementary to said mRNA molecule and a nucleic acid molecule homologous to said mRNA molecule, thereby identifying said desired protein or said desired nucleic acid molecule.

29. A method for identifying and selecting a desired protein or nucleic acid molecule comprising:

providing at least two candidate mRNA molecules, wherein at least one of said mRNA molecules contains at least one codon selected from the group consisting of a stop codon and a pseudo stop codon;

translating at least two of said candidate mRNA molecules to generate at least one translated protein;

linking at least one of said candidate mRNA molecules to its corresponding translated protein via a tRNA molecule to form at least one cognate pair, wherein at least one of said candidate mRNA molecules is connected to said tRNA molecule by a crosslinker;

identifying one or more of said cognate pairs based upon the properties of said translated protein or said mRNA molecule; and

identifying a molecule selected from the group consisting of one or more of the following: an mRNA molecule of said selected cognate pair, a nucleic acid molecule complementary to said mRNA molecule and a nucleic acid



molecule homologous to said mRNA molecule, thereby identifying said desired protein or said desired nucleic acid molecule;

providing an array of nucleic acids, wherein said nucleic acids are placed in a predetermined position;

hybridizing at least one of said cognate pairs onto said array;

reacting said at least one of said cognate pairs with one or more binding agents; and

selecting said desired nucleic acid molecule based upon a reaction or lack of a reaction to said one or more binding agents.

30. A method for identifying a desired protein or nucleic acid molecule comprising:

providing at least two candidate mRNA molecules, wherein at least one of said mRNA molecules contains at least one codon selected from the group consisting of a stop codon and a pseudo stop codon;

translating at least two of said candidate mRNA molecules to generate at least one translated protein;

linking at least one of said candidate mRNA molecules to its corresponding translated protein via a tRNA molecule to form at least one cognate pair,

wherein at least one of said candidate mRNA molecules is connected to said tRNA molecule,

wherein said tRNA molecule comprises a moiety which binds to a ribosome, accepts the peptide chain, and then does not act as a donor in the next transpeptidation,

wherein said moiety is selected from the group consisting of one or more of the following: a 2' ester on a 3' deoxy adenosine, an amino acyl tRNA<sub>ox-red</sub> and a puromycin;

identifying one or more of said cognate pairs based upon the properties of said translated protein or said mRNA molecule; and

identifying a molecule selected from the group consisting of one or more of the following: an mRNA molecule of said selected cognate pair, a nucleic acid molecule complementary to said mRNA molecule and a nucleic acid

molecule homologous to said mRNA molecule, thereby identifying said desired protein or said desired nucleic acid molecule.

31. A method for identifying a desired protein or nucleic acid molecule comprising:

providing at least two candidate mRNA molecules, wherein at least one of said mRNA molecules contains at least one codon selected from the group consisting of a stop codon and a pseudo stop codon;

translating at least two of said candidate mRNA molecules to generate at least one translated protein,

wherein said translating is performed *in situ*;

linking at least one of said candidate mRNA molecules to its corresponding translated protein via a tRNA molecule to form at least one cognate pair, wherein at least one of said candidate mRNA molecules is connected to said tRNA molecule ;

identifying one or more of said cognate pairs based upon the properties of said translated protein or said mRNA molecule; and

identifying a molecule selected from the group consisting of one or more of the following: an mRNA molecule of said selected cognate pair, a nucleic acid molecule complementary to said mRNA molecule and a nucleic acid molecule homologous to said mRNA molecule, thereby identifying said desired protein or said desired nucleic acid molecule.

32. A method for evolving a desired protein sequence comprising:

providing at least two candidate mRNA molecules, wherein at least one of said mRNA molecules contains at least one codon selected from the group consisting of a stop codon and a pseudo stop codon;

translating at least two of said candidate mRNA molecules to generate at least one translated protein;

linking at least one of said candidate mRNA molecules to its corresponding translated protein via a tRNA molecule to form at least one cognate pair, wherein at least one of said candidate mRNA molecules is connected to said tRNA molecule by a crosslinker;

identifying one or more of said cognate pairs based upon the properties of said translated protein or said mRNA molecule;

identifying a molecule selected from the group consisting of one or more of the following: an mRNA molecule of said selected cognate pair, a nucleic acid molecule complementary to said mRNA molecule and a nucleic acid molecule homologous to said mRNA molecule, thereby identifying said desired protein or said desired nucleic acid molecule;

providing a plurality of cognate pairs,

binding at least of said plurality of cognate pairs with one or more binding agents;

selecting said desired or protein nucleic acid molecule based upon a reaction or lack of a reaction to said one or more binding agents, thereby selecting a first desired cognate pair;

recovering said first desired cognate pair to generate a recovered cognate pair;

amplifying a first nucleic acid component of said recovered cognate pair;

producing a second nucleic acid component, wherein said second nucleic acid component comprises said first nucleic acid component with one or more variations;

producing a second protein by translating said second nucleic acid component;

linking said second protein with said second nucleic acid component to generate a second desired cognate pair; and

obtaining the desired protein sequence by re-selecting said second desired cognate pair based upon at least one desired property.

33. The method of Claim 32, wherein the step of selecting said first desired cognate pair comprises:

providing a first ligand with a desired binding characteristic;

contacting one or more of said first cognate pairs with said first ligand to generate unbound complexes and bound complexes;

recovering either the bound complexes or the unbound complexes;

amplifying at least one nucleic acid component of the recovered complexes;

introducing variation to a sequence of said nucleic acid component of said recovered complexes;

translating one or more second proteins from said nucleic acid components,

linking at least one of said second proteins with at least one of said second nucleic acid components to generate one or more second cognate pairs; and

obtaining the desired protein sequence by contacting said at least one of said second cognate pairs with at least one second ligand to select one or more of said second cognate pairs, wherein said second ligand is the same or different than said first ligand.

34. A method of forming a psoralen monoadduct on a nucleic acid, comprising:

providing a first nucleic acid and a second nucleic acid,

wherein said first nucleic acid and said second nucleic acid are substantially complementary to each other,

wherein said first nucleic acid comprises one or more uridine monoadduct targets, and

wherein said second nucleic acid comprises at least one pseudouridine

hybridizing said first nucleic acid and said second nucleic acid in the presence of psoralen to form a hybrid;

irradiating said hybrid with ultraviolet light, thereby forming said psoralen monoadduct on said first nucleic acid.

35. A method of producing a psoralen monoadduct or a crosslink, comprising:

providing a first nucleic acid and a second nucleic acid;

wherein said first nucleic acid and said second nucleic acid are substantially complementary to each other;

wherein said first nucleic acid comprises one or more uridine monadduct targets or crosslink targets and one or more uridine monoadduct non-targets or crosslink non-targets;

wherein said uridine monoadduct non-targets or crosslink non-targets are operable to be replaced with one or more pseudouridines;

replacing one or more of said uridine monoadduct non-targets or crosslink non-targets with pseudouridine;

hybridizing said first nucleic acid and said second nucleic acid in the presence of psoralen to form a hybrid;

irradiating said hybrid, thereby forming said psoralen monoadduct or said crosslink on said first nucleic acid on said targets, while protecting said nontargets.

36. A method for identifying and selecting a desired protein or nucleic acid molecule comprising:

providing at least two candidate mRNA molecules, wherein at least one of said mRNA molecules contains at least one codon selected from the group consisting of a stop codon and a pseudo stop codon;

translating at least two of said candidate mRNA molecules to generate at least one translated protein;

linking at least one of said candidate mRNA molecules to its corresponding translated protein via a tRNA molecule to form at least one cognate pair, wherein at least one of said candidate mRNA molecules is connected to said tRNA molecule by a crosslinker;

identifying one or more of said cognate pairs based upon the properties of said translated protein or said mRNA molecule;

identifying a molecule selected from the group consisting of one or more of the following: an mRNA molecule of said selected cognate pair, a nucleic acid molecule complementary to said mRNA molecule and a nucleic acid molecule homologous to said mRNA molecule, thereby identifying said desired protein or said desired nucleic acid molecule;

providing a plurality of cognate pairs,

binding at said plurality of cognate pairs with one or more binding agents; and

selecting said desired protein or nucleic acid molecule based upon a reaction or lack of a reaction to said one or more binding agents.

37. A vaccine produced by the method of claim 14.

38. A method for producing a SARS vaccine, comprising:

adding a sequence for the major histocompatibility complex class II (MHC-II) to cDNAs of a random mRNA library, wherein the MHC II-binding sequence permits the appropriate T-cell and ultimately, B cell response;

transcribing and translating said library, thereby producing proteins that correspond to their cognate MRNAs;

linking said proteins with said cognate mRNAs;

selecting one or more desired proteins from said library using a probe specific for the "S" epitope, wherein said probe is chemically linked to SPR membranes; and

selecting proteins that have high affinity for the anti-S antibody.